information about the properties defining the potential of KL_4 as surfactant additive.
The surface activity of the different peptides in lipid suspensions mimicking surfactant composition (DPPC/POPC/POPG 50:25:15 w/w/w), was analyzed in a captive bubble surfactometer (CBS). Only lipid suspensions containing KL_4 adsorbed to the air-liquid interface with kinetics comparable to those containing SP-B, with KL_4PQ and specially KL_2A_2 showing less activity. The interaction of the three peptides with membranes was analyzed by DSC, Laurdan fluorescence and ATR-FTIR. Perturbations induced by peptides on the structure and permeability of giant unilamellar vesicles (GUVs) were also analyzed and compared with the behavior of natural SP-B.
The results indicate that the extent of penetration of the peptides in bilayers and their ability to perturb membrane structure correlate with their different interfacial activity.

495-Pos Board B264
α-Synuclein Induced Membrane Curvature: What is the Significance of Negative Gaussian Curvature
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α-Synuclein (αS) is an intrinsically disordered protein that forms an amphipathic α-helix upon binding to lipid vesicles. Using Molecular Dynamics simulations we have recently shown that the protein induces an anisotropic curvature field in the phospholipid bilayer when bound to a membrane. First, as is known to be characteristic of amphipathic helices, αS induces positive mean curvature (a hill). More interestingly, our simulations suggested unexpected complexities in the induced Gaussian curvature field. Our current efforts, described here, explore the physical principles that dictate these membrane curvature features, again using large-scale simulations. Our focus has been on how three physical aspects of the protein (helix length, flexibility, and hydrophobicity) lead to these complex curvature fields. In addition, we explore the relationship between negative Gaussian curvature and membrane tubulation.

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Three Mutations in the BIN1 N-BAR Domain Impair Membrane Curvature Generation via Different Mechanisms
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Cellular signaling and membrane transport are tightly coupled to membrane curvature. Bin/Amphiphysin/Rvs (BAR) domain-containing proteins are key players in regulating membrane shapes. Particularly, BIN1, an N-BAR domain containing protein is believed to be essential in initiating and maintaining plasma membrane invaginations (T-tubules) in muscle cells. Three mutations, K35N, D151N and R154Q, have been discovered so far in the BAR domain of BIN1 in patients with centronuclear myopathy (CNM), where biogenesis of T-tubules is compromised. However, the molecular mechanisms remain elusive. Here, we demonstrate that all CNM-related mutations lead to loss of membrane tubule generation in C2C12 myoblasts. We also use several in vitro assays to characterize the differences among BIN1 N-BAR variants with respect to membrane association affinities and membrane tubulation potential. We find that the mutations-D151N and R154Q in the BAR domain reduce membrane binding affinities and membrane-bound protein densities especially on highly curved liposomes, whereas the K35N mutation in the N-terminal inserting helix binds membranes similarly as the wild type protein (WT). We observe that both WT and K35N can deform vesicles into ~ 30nm wide tubules and tubulation capacity is dependent on membrane surface charge and protein concentration. Contrarily, D151N and R154Q show limited ability to induce membrane curvature. Equilibrium tube-pulling force measurements report on mechanical effects of proteins on established membrane tethers and suggest that only WT and K35N oligomerize on membrane tethers. R154Q barely causes a tether pulling force reduction, consistent with the observation that R154Q shows the weakest membrane binding affinity. We hypothesize that the D151N mutation impairs protein oligomerization interface and is deficient in membrane-mediated protein lattice formation, thus defective curvature induction. Taken together, our results provide new insights into the membrane-involved pathophysiological mechanisms leading to human disease.

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Characterization of Antimicrobial Methylated Tryptophan Retro Lactoferricin Peptides by Solid State NMR and Fluorescence Spectroscopy
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Lactoferricin B (LfB) is a 25-residue peptide from the N-terminus of bovine lactoferrin with broad-spectrum antimicrobial activity. A hexapeptide has been found that retains antimicrobial activity (LfB6: RRWQWR-NH2) (Tomita, et al., *Acta Paediatr Jpn*, 1994, **36**:585-91). Previous work in our lab has shown that reversing the sequence (Retro LfB6: RWQWRR-NH_2), amino-acylation, or tryptophan-methylation (MeTrp) increase antimicrobial activity. We now compare the effects of Trp-methylation at position 2 (RMeWQWRR-NH_2; Retro LfB6 MeTrp2) versus position 4 (RWQMeWRR-NH_2; Retro LfB6 MeTrp4), and of N-acylation of each peptide with a C6-fatty acid tail. Selective deuteration of the MeTrp residues permits analysis of the peptides by solid-state ^{2}H NMR spectroscopy in oriented bilayers. We find that the antimicrobial activity of peptides with MeTrp at position 2 is reduced compared to position 4, except in the case C6-Retro MeTrp2 which exhibits greater activity towards *E. coli*. ^{31}P NMR spectra of oriented samples composed of DMPC and DMPC:DMPG (3:1), to mimic mammalian or bacterial cell membranes, respectively, reveal that the bilayer remains intact when exposed to the peptides. This suggests the mechanism for antimicrobial activity does not involve large scale membrane perturbation. ^{2}H NMR spectra reveals that the MeTrp in the acyl and non-acyl MeTrp2 peptides is less well-aligned on the surface compared to the MeTrp4 peptides. Partitioning assays confirm weaker binding for Retro MeTrp2 peptides compared to MeTrp4. Fluorescence spectroscopy shows that, although the spectra of the Retro MeTrp4 and MeTrp2 peptides are similar, the emission max for the Trp and MeTrp residues in acyl and non-acyl Retro MeTrp2 peptides are blue-shifted (lower max) compared to the Retro MeTrp4 peptides, indicating a less polar environment.

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Dissociation of the K-Ras4B/PDEδ Complex Upon Contact with Lipid Membranes: Membrane Delivery Instead of Extraction
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K-Ras4B is a small GTPase that plays a critical role in human cancer cell biology. Selective membrane localization and clustering of K-Ras4B into microdomains are mediated by its polybasic farnesylated C-terminus. The importance of the subcellular distribution for the signaling activity of K-Ras4B became apparent from recent *in vivo* studies, showing that the delta subunit of cGMP phosphodiesterase (PDEδ), which possesses a hydrophobic prenyl-binding pocket, is able to function as a potential binding partner for farnesylated proteins [1], thereby leading to a modulation of the spatiotemporal organization of K-Ras [2]. Even though PDEδ has been suggested to serve as a cytosolic carrier for Ras, the functional transport mechanism still remains largely elusive.
In this study, the effect of PDEδ on the interaction of GDP- and GTP-loaded K-Ras4B with neutral and anionic model biomembranes has been investigated by a combination of different spectroscopic and imaging techniques. The combined results show that PDEδ is not able to extract K-Ras4B from membranes. Rather, the K-Ras4B/PDEδ complex formed in solution turned out to be unstable in the presence of heterogeneous membranes, resulting in a release of farnesylated K-Ras4B upon membrane contact. With the additional observation of enhanced membrane affinity for the K-Ras4B/PDEδ complex, a molecular mechanism for the PDEδ-K-Ras4B-membrane interaction could be proposed [3]. This includes an effective delivery of PDEδ-solubilized K-Ras4B to the plasma membrane, dissociation of the K-Ras4B/PDEδ complex upon plasma membrane contact, and finally membrane binding of released farnesylated K-Ras4B that leads to K-Ras4B-enriched microdomain formation as a putative signaling platform.
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499-Pos Board B268
Anionic Lipids are not Essential for Membrane Tubulation by α-Synuclein
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α-Synuclein (α-syn) is an abundant protein of ill-defined function enriched in the presynaptic terminals of neurons. The presence of aggregated or amyloid α-syn in the brain is a hallmark of Parkinson's disease and its conformation and aggregation kinetics are modulated by membranes. In our work, we aim to understand how α-syn association influences the structure and properties